Qazvin University of Medical Sciences

Faculty of Paramedical Sciences

Thesis submitted for the degree of M.Sc. in Medical Biotechnology

Title:

Study of nonstructural protein 4 (NSP4) gene expression of RF rotavirus with removal of the diarrhea-inducing domain in E. coli BL-21 DE3 cells

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Abstract

Aims: Rotavirus is the leading cause of life-threatening diarrhea among children below five years of age resulting in approximately 453000 deaths worldwide. Rotavirus nonstructural protein 4 (NSP4) is the first identified viral enterotoxin causing age-dependent diarrhea. Amino acids 114-135 of NSP4 are known to make the diarrhea-inducing region of this glycoprotein. The aim of this study is evaluation of NSP4 expression in *E. coli* before and after removal of the diarrhea-inducing domain.

Materials and methods: In this study, we used splicing by overlap extension (SOEing) PCR to remove the diarrhea-inducing sequence from NSP4 cDNA and cloned both the full-length (FL-NSP4) and the spliced (S-NSP4) cDNA amplicons into pET-32c and pGEX-6P-2. Expression of the recombinant proteins were then evaluated in *Escherichia coli* BL21 (DE3) by Western blotting analyses.

Results: For FL-NSP4, protein expression was detected for the strain containing pGEX:FL-NSP4 but not for the one carrying pET:FL-NSP4, and hourly sampling up to 3 hours showed that the protein production of positive samples decreased by time, probably due to toxicity and/or protein degradation. In contrast, detection of S-NSP4 was positive for the pET:S-NSP4 strain but negative for the one carrying pGEX:S-NSP4; and Western blots showed that the amount of this protein was relatively low, possibly because of toxicity.

Conclusion: Stability, functionality, and toxicity of recombinant proteins can be affected by the constitutional fusion-tags in expression vectors most probably because of their impact on the folding of the target proteins. These tags can also affect expression levels. Production of NSP4 without the diarrhea inducing domain shows promise for immunological and vaccine studies in the future.